An Evaluation of Novel Lipid-Enveloped Nanoparticles for Adjuvant and Antigen delivery for an HIV Vaccine: Stepping from Laboratory into Potential Markets

by

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Abstract

Enormous effort has been devoted to the development of a vaccine against human immunodeficiency virus (HIV). The purpose of this paper is to evaluate the technological and economical aspects of a potential vaccine designed by Professor Irvine's group. Lipid-enveloped virion-sized nano-particles with a biodegradable polymer core are used as synthetic pathogens to deliver HIV specific antigens and adjuvants. The nano-particles are designed to display multiple copies of the antigen on their surfaces and to elicit humoral immunity response. Topics such as patent ability, obtaining an FDA licensure, storage, cost of manufacturing, and supply of the vaccine are explored. A business model for commercialization of the vaccine is outlined, and some possible future business opportunities for the nano-particles are discussed.

Acknowledgments

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I would like to thank my amazing friends and Craig, who made my undergraduate and graduate journey at MIT very enjoyable. Thank you for helping me shape this experience into something I will never forget.

I am grateful for having such amazing parents who have provided me with everything I needed to succeed. To my sister, Ida, who has always been my role model and have encouraged me all throughout my life. I could have not done this without the unconditional love and support of my family. This thesis is dedicated to them.

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1 Introduction and Background

1.1 The Global Need for an HIV Vaccine

The United Nations Joint Program on HIV/AIDS (UNAIDS) estimates that AIDS has killed more than 25 million people since it was first discovered in 1981.[1] The number of people living with HIV in 2008 was reported to be 33.4 million, of whom 22.4 million live in Sub-Saharan Africa.[2] In 2008, 2.7 million people were newly infected with HIV and 2.0 million died due to AIDS.[2] Although significant progress has been achieved in lowering the annual number of AIDS related deaths and in preventing new HIV infections, the number of people living with HIV continues to increase.

AIDS-related illnesses have been one of the leading causes of death globally and are projected to continue as a major global cause of premature mortality in the coming decade. It has been estimated that with a high efficacy vaccine, global needs are in the order of 690 million full immunization courses.[3] Development of an effective vaccine could potentially save the lives of millions people around the world and help to end the pandemic.

1.2 HIV Virus Structure

Human Immunodeficiency Virus (HIV) is a retrovirus that causes acquired immunodeficiency (AIDS), a condition in which the immune system begins to fail, leading to life-threatening opportunistic infections. The virus is about 80-120 nm in diameter and roughly spherical. It is composed of an inner core and a viral membrane. The inner core contains two identical, non-complementary strands of RNA (viral genome) and three enzymes including reverse transcriptase, protease and integrase as shown in Figure 1.[4] The inner core is surrounded by a conical capsid comprising the viral protein p24, which protects the nucleic acid core. The capsid can also readily absorb to host cell surfaces to introduce the viral genome.[5]

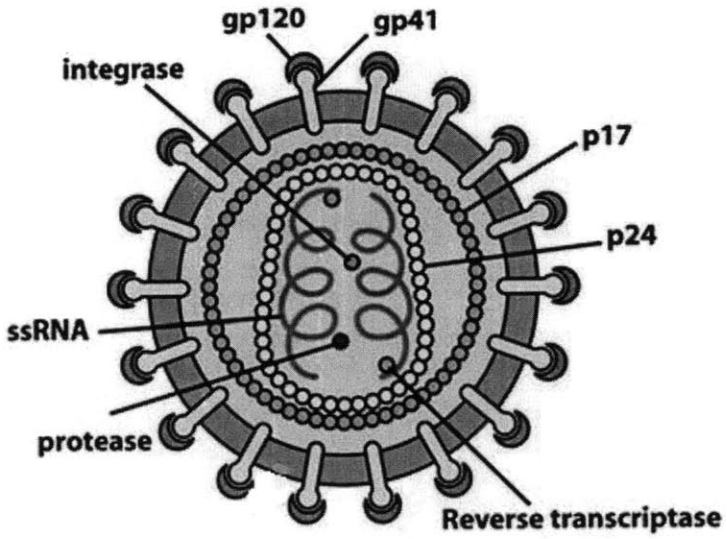

Figure 1: The HIV virus structure.[4]

The HIV viral membrane (envelope) encloses the particle and is made of glycoprotein subunits that are exposed as spikes. Gp120 is the surface glycoprotein that is bound to the virus by a transmembrane glycoprotein (gp41). The gp120 and gp41 form a non-covalent complex, and a protein called P17 lines the inner surface of the membrane. Both the gp120 and gp41 are capable of variations, which produce different strains of the virus, and the number of strains increases as the HIV infection progresses.[5]

1.3 Mechanism of Virus Infection

HIV infections may be caused by one of two retroviruses, HIV-1 or HIV-2. HIV-1 has caused a worldwide epidemic, but HIV-2 tends to be limited to West Africa. The virus can target any cell carrying the CD4 protein on its surface (CD4+ cells). Different strains of HIV target different cells, but T cells and macrophages, which are both CD4+, are important targets for the HIV virus. The first step in the virus life-cycle is binding to the cell CD4 receptor, after which the

spike proteins bind to the co-receptors, and the viral membrane fuses with the host cell membrane. HIV most commonly uses CCR5 or CXCR4 as a co-receptor to enter its target cells. CCR5 and CXCR4 are both chemokine receptors that are encoded in humans by the genes CCR5 and CXCR4 respectively.[6]

Once the virus enters the target cell, the viral RNA is transcribed into provirus DNA by reverse transcriptase enzyme. The provirus DNA is integrated into the genome of the infected cell.[7] After the virus has infected the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function or the virus becomes active and replicates, and a large number of virus particles that can then infect other cells are liberated. HIV infection damages CD4+ T cells, and suppresses the function of the infected cells resulting in diminished cell-mediated immune response.

The HIV virus has a high genetic variability that makes it difficult to generate anti-body responses that can control the virus. The high genetic variability of HIV is a result of its fast replication cycle (about 10^{10}-10^{12} virions every day)[8], high mutation rate (approximately $3*10^{-5}$ per nucleotide base per replication cycle)[9] and recombinogenic properties of reverse transcriptase. HIV mutations allows the virus to evade immune responses; An HIV infected person becomes progressively more susceptible to opportunistic infections and eventually develops Acquired Immunodeficiency Syndrome (AIDS).[5]

2 HIV Current Treatment Options

2.1 Post- Exposure Prophylaxis (PEP)

Post-exposure prophylaxis (PEP) medications are antiretroviral medications (ARVs) given after an HIV or suspected HIV exposure to decrease the likelihood of HIV infection from the exposure. Thousands of people worldwide are exposed to HIV accidentally while performing their work duties. PEP medications are generally associated with occupational exposure, such as needle sticks for healthcare workers, and are widely considered as an integral part of overall strategy for preventing HIV transmission.[10] They should be taken within the first hours of the exposure and the dose is usually multiple times per day for about four weeks.

The combination of medications given depends on the degree of exposure and the HIV status of the source of exposure. In some cases, 2 or 3 or even more PEP medication combinations are used.[10] There are some concerns associated with the use of PEP. In some people, PEP medications can cause serious side effects such as liver toxicity.[11] Also, poor adherence to the medication can result in development of drug-resistant HIV strains. The drugs have to be taken several times a day for at least 30 days and the costs of medication is between $600 and $1,000.[12]

2.2 Highly Active Antiretroviral Therapy (HAART)

Highly Active Anti-Retroviral Therapy (HAART) consists of three or more highly potent anti-HIV drugs. HIV virus changes to avoid detection, and if only a single drug therapy is used, it might be successful for a while, but drug-resistant strains will often arise in the patient. However, it is unlikely that the HIV genome would mutate such that it can resist three separate drug treatments at once.[13] The antiretroviral drugs each inhibit a phase in the retrovirus life-cycle and are divided into several categories. For HAART therapy, usually combinations consisting of at least three drugs belonging to at least two types of antiretroviral agents are used.

Nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI), inhibit the activity of reverse transcriptase, a viral DNA polymerase enzyme that retroviruses need to replicate.[14] Protease inhibitors (PIs) inhibit the activity of HIV-1 protease, an enzyme used by the virus to cut polyproteins for final assembly of new virions.[15] Another class of antiretroviral drugs is entry inhibitors which interfere with the binding, fusion and entry of an HIV virion to a human cell.[16] Integrase inhibitors block the action of integrase, a viral enzyme that inserts the viral genome into the DNA, and by doing so, they prevent replication and further spread of the virus.

Raltegravir was the first integrase inhibitor which obtained FDA approval in October 2007, but there are several other drugs currently under clinical trials.[17] Maturation inhibitors target the Gag structural polyproteins of HIV and inhibit the production of HIV capsid protein; one of the last steps in the viral lifecycle within an infected human cell. There are currently no approved FDA drugs in this class, but the two which are going through clinical trials are Vivecon and Bevirimat.[18,19]

HAART medications drive HIV levels (viral load) below detection in blood, semen and vaginal secretions and increase the number of CD4+ T cells. As a result they contribute to fewer opportunistic infections (OIs) and HIV-associated cancers and can overall improve the quality of life for people with HIV/AIDS.[20] However, they can have serious side-effects in some people such as peripheral neuropathy (nerve damage) and lipodystrophy (fat redistribution).[20] Antiretroviral treatment must be taken every day for life once started and every missed dose can increase the risk of the drug becoming ineffective. There can be effective in less than fifty percent of the patients due to several reasons such as poor adherence, medication intolerance/side effects, prior ineffective anti-retroviral therapy, or infection with a drug-resistant strain of HIV.

Moreover, HAART medications neither cure nor uniformly remove all the symptoms. They are also expensive and the majority of infected individuals do not have access to them.[21] Table 1 shows the percentage of people who have access to antiretroviral drugs. Overall, only 42% of total people in need of antiretroviral drugs are receiving them.

Region (lower- and middle-income countries)	Antiretroviral therapy coverage	Estimated number of people receiving antiretroviral therapy	Estimated number of people needing antiretroviral therapy
Sub-Saharan Africa	44%	2,925,000	6,700,000
Eastern and Southern Africa	48%	2,395,000	5,000,000
Western and Central Africa	30%	530,000	1,800,000
Latin America and the Caribbean	54%	445,000	820,000
Latin America	55%	405,000	740,000
The Caribbean	51%	40,000	75,000
East, South and South-East Asia	37%	565,000	1,500,000
Europe and Central Asia	23%	85,000	370,000
North Africa and the Middle East	14%	10,000	68,000
Total	42%	4,030,000	9,500,000

Table 1: Estimated number of adults and children (combined) receiving antiretroviral therapy and needing antiretroviral therapy and percentage coverage in low- and middle income countries by region (December 2008)[21]

Although there is still not enough supply of antiretroviral medications to meet the increasing needs, the percentage of people receiving the drugs has increased over the years as shown in the Figure 2. Another concern with the use of HAART is the cost of the treatment. Figure 3 shows the median annual cost (in US dollars) of first-line antiretroviral drug regimens in low-income countries from 2004 to 2008. First regimen drugs are ones with a high efficacy and low side-effect profile, and typically include two nucleoside analogue reverse transcriptase inhibitors (NARTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (nNRTI).[21]

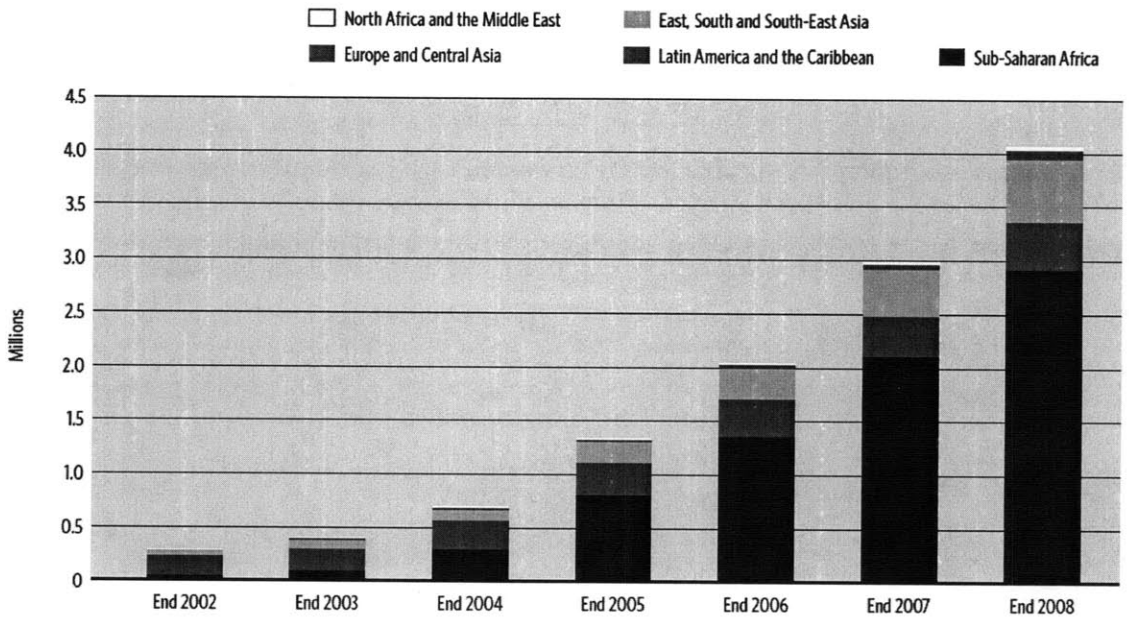

Figure 2: Number of people receiving antiretroviral therapy in low- and middle-income countries, by region, 2002–2008. [21]

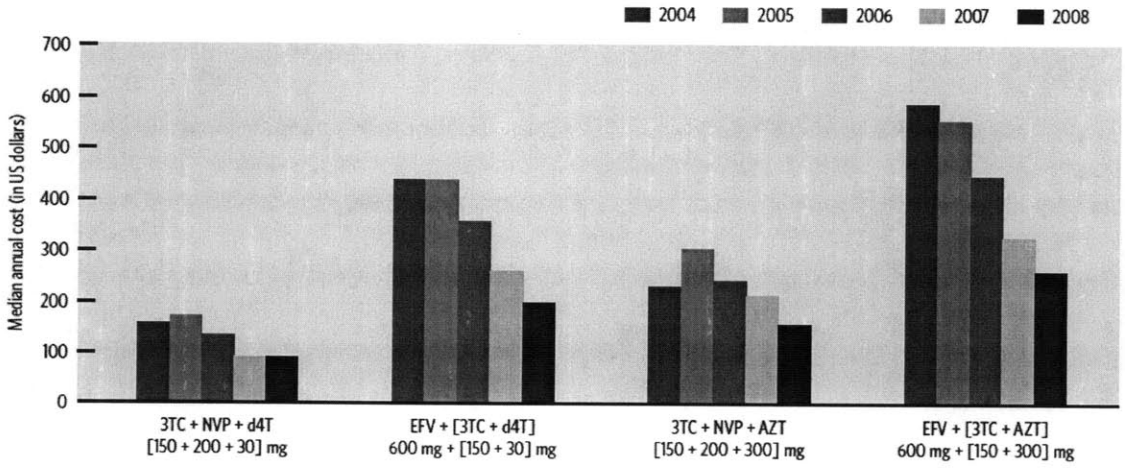

Figure 3: Median annual cost (in US dollars) of first-line antiretroviral drug regimens in low-income countries by year, (2004–2008).[12] Note that AZT, 3TC, d4T (Stavudine) are nucleoside analog reverse transcriptase inhibitor (NARTI) while EFV (Efavirenz) and NVP (Nevirapine) are non-nucleoside reverse transcriptase inhibitor (nNRTI).[22]

The data in Figure 3 indicates a 30-68% decrease in costs from 2004 to 2008, which could help with increasing the availability of the drug. The general trend of decrease in price might be due

to increasing transaction volumes, competition between growing number of products prequalified by WHO and favorable pricing policies by pharmaceutical companies.[21] Despite the trend of decrease in prices, the cost of these medications is still high for people in developing countries and the treatments are not easily affordable.

3 Types of HIV Vaccine

Enormous effort has been devoted to the development of a vaccine against human immunodeficiency virus (HIV). Despite the tremendous amount of economic and intellectual efforts, no effective vaccine has yet been introduced to the market. The main challenges in the design of an effective vaccine against HIV are the virus ability to evade the immune system, its high genetic variability, high rate of mutation and unique biology of replication.[23] The efforts to develop a vaccine have been focused on two main strategies: eliciting a cellular immune response or a humoral immune response. In this section, these two different approaches are discussed.

3.1 Vaccines Inducing Cellular Immunity

Cellular immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated natural killer (NK) cells, and cytokines in response to an antigen (Figure 4).[24] The goal of a vaccine based on cellular immunity is to protect the body by activating antigen-specific cytotoxic T-lymphocytes (CTLs) that are able to recognize the epitopes of foreign antigen bound to the cell membrane glycoproteins (the major histocompatibility complex (MHC) class I and class II) and destroy the infected cells.[25]

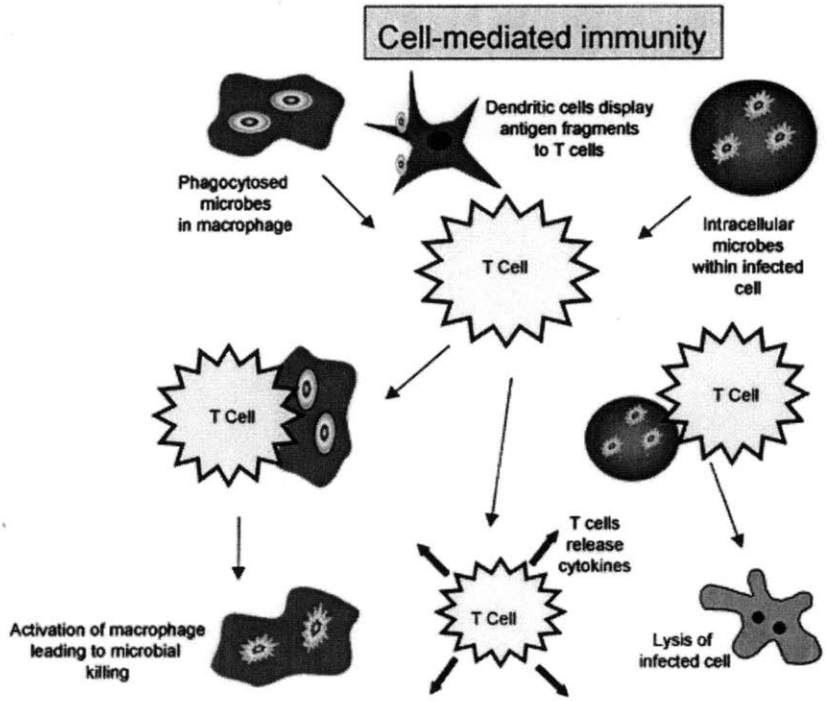

Figure 4: Cell mediated immunity is mediated by T cells, with dendritic cells playing important roles in antigen presentation. Various methods by which T cells function include activating macrophages to kill phagocytosed microbes, releasing cytokines and directly destroying infected cells.[24]

The two parts of the cellular immunity important in the design of an HIV vaccine are T-helper (primarily Th1) and the cytotoxic T-lymphocyte responses. For a cytotoxic T-cell to be able to eliminate a virally-infected cell, it requires the biochemical help of a Th1 helper T-cell. The focus of the vaccines based on cellular immunity has been to deliver antigens into the cytosol of the cells to induce MHC class I-restricted CTL response. The delivery of the antigen to the cell has mainly been done by introducing the gene encoding the antigen. The cells to which these antigens are delivered should be either antigen presenting cells or ones capable of transferring the antigen to professional antigen presenting cells.[26]

One of the motivations to use a CTL approach for HIV vaccine design is that T-cells can recognize the internal (as well as external) epitopes of the HIV virus. The internal HIV proteins are more conserved than some regions of the external envelope proteins since the virus needs to have some conserved regions associated with its basic function and structure.[26] The higher degree of conservation of non-surface proteins such as Gag and Pol compared with surface proteins such as Env, suggests that a vaccine based on the cellular immunity might be able to detect more diverse viral strains and produce a broader immune response. However, mutations have been observed in CTL epitopes as well as antibody epitopes.[26] One limitation of a vaccine based on cellular immunity is that it requires an infection to be underway to be cleared by the immune system. T-cells play a role only after a cell has been infected and do not prevent the entry of the virus into the cells.

3.2 Vaccines Inducing Humoral Immunity

Humoral immunity involves the production of antibody (immunoglobulin) molecules in response to a specific antigen and is mediated by B-lymphocytes (Figure 5).[24] These antibody molecules circulate in the blood and are secreted into mucosa. They are effective against bacteria, bacterial toxins, and viruses prior to these agents entering cells.[27]

The only targets for neutralizing antibodies against HIV-1 are the surface gp120 and trans-membrane gp41 envelope glycoproteins (Env). These interact with the receptors on the target cell and, by fusing the viral and cell membranes, allow the virus to gain entry into cells. Antibodies would neutralize the virus by binding to the glycoprotein and preventing the virus to enter cells such as CD4+ T cells.[28]

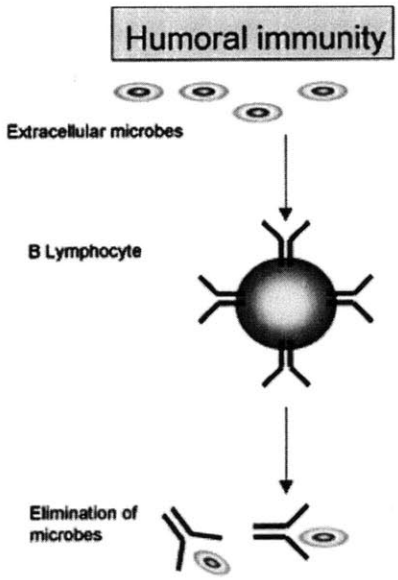

Figure 5: Humoral Immunity is mediated by antibodies that are produced by B Lymphocytes. Secreted antibodies bind to viruses to assist in their elimination.[24]

HIV-1 tends to elicit production of neutralizing antibodies (NAbs) against variable regions of the virus, whereas NAbs that target conserved regions on the virus are rare.[29] An effective vaccine would be one that generates broadly neutralizing antibodies (bNAbs) which can neutralize or inactivate a wide spectrum of the viral isolates representing the major genetic clades of HIV-1 strain. [30] In the design of the immunogens for antibody-based vaccines, the structure of the HIV-1 envelope has been studied, and conserved epitopes that are recognized by bNAbs have been identified. These epitopes, which are conserved among different strains, are more likely to produce cross-reacting antibodies. [29,30]

The CD4 binding site (CD4bs) of the virus has shown to be highly conserved among different strains and is recognized by bNAbs such as b12, VRC01 and HJ16. This binding site is an important target for bNAbs since the binding of gp120 to CD4 on target T-cells is an essential step in early infection of the cell with the virus.[29] The CD4bs is within the core of gp120 and is not easily accessible by the antibody molecules. The virus has a dense outer layer of sugar molecules, which helps it evade detection by antibodies.[29]

The broadly neutralizing antibody 2G12 also primarily interacts with the outer domain of gp120. The 2G12 epitope consists mainly of mannose residues but it is not directly associated with the receptor-binding sites on the protein. 2G12 can neutralize the virus and prevent infection of the cell by inhibiting the interaction of HIV-1 with its cell surface binding sites.[31] Since the outer domain of gp120 is poorly immunogenic, research is being done on isolating the epitopes of the outer domain construct that expose the b12 and 2G12 binding sites.[29]

Another highly conserved epitope of the envelope protein is the membrane-proximal external region (MPER) of gp41. The broadly neutralizing antibodies 2F5, 4E10 and Z13e1 are directed against linear epitopes mapped to the MPER.[32] Also, there are epitopes that reside in the variable loops 1, 2, and 3 of the gp120 which are recognized by PG9 and PG16 broadly neutralizing antibodies.[33] Figure 6 shows the location of the different bNAbs epitopes on the HIV viral spike.[34]

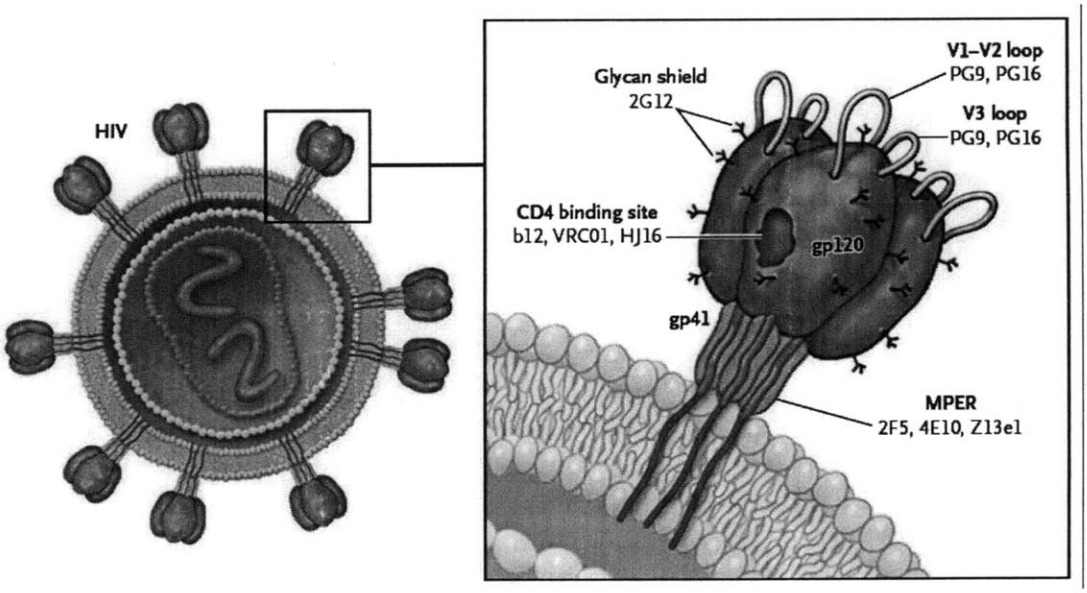

Figure 6: Targets for Potential Vaccines on the Trimeric HIV Envelope Spike Glycoproteins (gp120 and gp41). Broadly neutralizing antibodies have been identified that target the CD4 binding site (b12, VRC01, HJ16) on glycoprotein 120, the membrane proximal external region (2F5, 4E10, Z13e1) of gp41, the glycan shield (2G12), and epitopes that reside in the variable loops on gp120 (PG9, PG16).[34]

Different technologies such as nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), and surface plasmon resonance (SPR) has shown that MPER is immersed in the viral membrane, enabling it to prevent detection by immune system, and it has a hinge in the middle, which provides flexibility and helps it attach to white blood cells.[35] Immunogen design based on the MPER has been supported by a recent study in which it was shown that 4E10 and 2F5 can protect against mucosal simian-human immunodeficiency virus (SHIV) challenge in macaques.[36] The MPER epitope is the antigen used in the Irvine lab's vaccine design.

Developing a vaccine based on humoral immunity has the advantage that the broadly neutralizing antibodies can potentially neutralize the virus and prevent it from infecting the cells. The challenges with this vaccine approach are the high variability of the envelope glycoprotein in amino acid sequence, glycosylation pattern and the low immunogenicity of the conserved regions which are attractive targets for a vaccine design.

4 Vaccine Candidates/ Clinical Trials

There are several ongoing studies of HIV candidate vaccines worldwide. Many of these vaccines are still in pre-clinical stage or phase I of clinical trials. This section is devoted to the discussion of the major candidate HIV vaccines and the results of their clinical trials.

4.1 AIDSVAX B/B and AIDSVAX B/E

Two of the first phase III preventive HIV vaccine trials were the AIDSVAX B/B trial (United States, Canada) in men who have sex with men (MSM), and the AIDSVAX B/E trial (Thailand) in injection drug users (IDU).[37] AIDSVAX was developed and trialed by the VaxGen company. The vaccine was made with genetically engineered proteins similar to gp120, a protein unique to HIV's surface. As an adjuvant, Alum was combined with the recombinant gp120 to boost the vaccine's effectiveness.

The AIDSVAX B/B vaccine was based on two strains of HIV clade B which is predominant in North America and Europe. Since the vaccine was not made with the actual virus, it was safe and could not cause HIV infection. The hope of the trial was that the vaccine would result in production of antibodies which can prevent HIV infection. However, the results of the study completed in 2003 showed that the vaccine was ineffective at preventing HIV and did not raise particularly high levels of anti-gp120 antibodies in most volunteers.[37,38]

4.2 STEP Study/ Phambili

The STEP study is a more recent phase IIB HIV vaccine trial which was managed by HIV Vaccine Trials Network (HVTN). The vaccine was developed by Merck & Co. It was an adenovirus synthetically modified to contain proteins from HIV such as Gag, Pol, and Nef. The trial group included all HIV-negative men and women who were identified as a high-risk population. The hope was that the adenovirus vector would carry the HIV-1genes into the cells

and induce a cell-mediated immune response. This study which began in 2005, was halted in 2007 when the vaccine was proved to be ineffective.[39]

Additionally, the results of STEP study suggested an increase in HIV infection in people who received the vaccine and had pre-existing adenovirus antibodies compared to ones that did not receive the vaccine. The Phambili trial was a trial in South Africa using the same vaccine which was discontinued at the same time. Almost everyone enrolled in the STEP study had received the full course of the vaccine, but no one in the African trial had been entirely vaccinated.[39]

4.3 RV144

RV144 was the largest Phase III HIV Vaccine Trial conducted in humans, which involved more than 16,000 volunteers in Thailand. The trial tested a "prime-boost" combination of two vaccines: ALVAC HIV vaccine (the prime), and AIDSVAX B/E vaccine (the boost). ALVAC-HIV consists of a viral vector and genetically engineered versions of three HIV genes (*Env*, *Gag* and *Pro*). The viral vector is a disabled form of a bird virus called canarypox, which cannot grow or cause disease in humans. AIDSVAX B/E, as mentioned above, is composed of genetically engineered gp120.[40] These two vaccines were designed to stimulate both humoral and cell-mediated immunity to HIV-1 and the vaccine combination was based on HIV strains that commonly circulate in Thailand.

The trial involved a total of six immunizations over six-months: four immunizations with ALVAC-HIV and two with AIDSVAX B/E given at the same time as the last two ALVAC-HIV injections. The test was designed to assess the vaccine's ability to prevent HIV infection, as well as its ability to reduce the amount of HIV in the blood of those who became infected after enrolling in the trial.[41] The study was carried out by the US army and the Thai government over 7 years. It began in October 2003, vaccination was ceased in July 2006, and efficacy findings

publicly released in September 2009. The results of the study are shown in Figure 7. The data suggested that the vaccines were safe and resulted in a 31% prevention of HIV infection.[41] Although this has been the first positive news in HIV/Aids vaccine, further studies are being done to understand how the vaccine regimen reduced the risk of HIV infection.

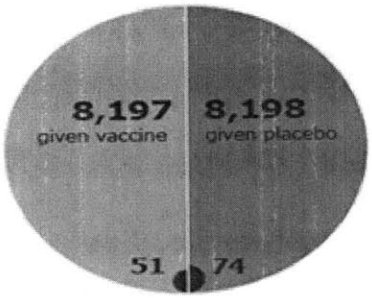

Figure 7: The results of RV144 trial. 74 people receiving the placebo acquired HIV compared to 51 people receiving the vaccine. (total number of people, all HIV-negative men and women age 18-30, 16,395) [41]

4.4 HVTN505

Another recent study that started in 2009 is the HVTN505 trial. This study uses a DNA prime/rAd5 boost vaccine regimen developed by the Vaccine Research Center at the National Institutes of Health (NIH), and is managed by HIV Vaccine Trail Network (HVTN). The vaccine is not expected to prevent HIV infection but to decrease the viral load of those people who become infected with HIV and the results will be helpful in development of T-cell-based vaccines. The study population is limited to U.S. men who have sex with men (MSM), are circumcised and do not have antibodies to Adenovirus type 5.[42] Table 2 shows the list of vaccine efficacy trial done to date.[43]

Vaccine Concept	Developer	Study	Status
Monomeric gp120 (B/B, B/E)	VaxGen	VAX 003, VAX 004 (phase III)	Completed in 2003
rAd5-Gag/Pol/Nef	Merck	HVTN 502 STEP, HVTN 503 Phambili (phase IIb)	Stopped in 2007
ALVAC (vCP1521) prime, gp120 (B/E) boost	Sanofi, VaxGen (GSID)	RV 144 (phase III)	Completed in 2009
DNA prime, rAd5 boost Gag/Pol/EnvA/EnvB/EnvC	NIH VRC	HVTN 505 (phase II)	Started in 2009

Table 2: HIV-1 vaccine efficacy studies.

5 The Novel Nano-Particle HIV Vaccine

The vaccine developed by the Irvine group is an antibody-based vaccine which elicits humoral immunity response. Lipid- enveloped virion-sized nano-particles with a biodegradable polymer core are used as synthetic pathogens which deliver HIV specific antigens and adjuvants. The structure of these nano-particles, the nature of the antigen and the adjuvant, and the method to produce the particles are outlined in the following sections.

5.1 Lipid-Enveloped Polymer Core Nano-particles

In the HIV vaccine proposed by the Irvine group, nano-particles are used both as delivery systems and adjuvants. One important property of the nano-particles is that multiple copies of the antigen can be displayed on their surfaces which is optimal for B cell activation. It has been shown that antigen organization influences the B cell response such that organized arrays of antigens can efficiently cross-link with B cell receptors and cause a strong activation signal.[44] Also, nano-particles compared to microparticles have a larger surface area to volume ratio for antigen adsorption which allows for a higher antigen:polymer ratio.[45] The size of the particles affects their immunogenicity, and in a previous study on polylactic acid (PLA) particles, it has been shown that smaller particles (<5 micrometer) are more immunogenic than larger ones and induce a higher antibody production. The higher immunogenicity is suggested to be due to an enhance uptake of the smaller particles into lymphatics and a more efficient uptake by Antigen Presenting Cells (APCs).[46]

The Irvine nano-particles have a poly (D,L-lactic-co-glycolic) (PLGA) core. Other polymers such as poly(methyl methacrylate) have been studied by other groups before; however, these polymers degrade very slowly in vivo.[46] One advantage of using PLGA is that this polymer is already used in clinical applications such as resorbable sutures, and scaffolds for tissue

engineering.[47] The PLGA high biocompatibility and biodegradability makes it ideal for vaccine delivery applications. The polymer undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These monomers are by-products of various metabolic pathways in the body; hence there is minimal systemic toxicity associated with PLGA degradation in the body.[48]

The nano-particles used in the vaccine have dimensions comparable to pathogens that the immune system has evolved to combat, and their lipid shell composition is similar to the envelope of the human immunodeficiency virus HIV-1. The three lipids used for the lipid envelop are 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethyleneglycol)2000] (maleimide-PEG2k-PE).[49] This fluid lipid bilayer incorporates lipid like adjuvants, namely Monophosphoryl Lipid A (MPLA), which enhance the potency of vaccine particles.

The specific HIV antigen is attached to the surface via maleimide-PEG2k-PE which has a functionalized head group displaying a sulfhydryl-reactive maleimide ester. Protein antigens are purified and modified to react with the sulfhydryl group and bind to the surface. It has been shown that when an antigen is associated with nanoparticles or microparticles, a stronger immune response is elicited compared to soluble antigen.[45]

Also, the lipid shell might play a role in the antibody neutralizing effect since it has been shown that broadly neutralizing antibodies such as 4E10 and 2F5 have lipid-binding properties.[50] Recent data supports that 2F5 and 4E10 binding to HIV-1 involves both viral lipid membrane and gp41 membrane proximal epitopes.[51]

5.2 Antigen: Membrane Proximal External Region

In developing an HIV vaccine unlike traditional vaccines, live but attenuated or inactive whole organism cannot be used due to safety concerns.[52] The target for any antibody based vaccine would be the HIV virus envelope protein. In the Irvine vaccine nano-particles, the antigen displayed on the surface is only a portion of the HIV gp41 spike protein called the Membrane Proximal External Region (MPER). This region includes the last 24 C-terminal amino acids of the gp41 ectodomain.[53]

There are two properties of MPER which make it a very attractive antigen to be used for the Irvine vaccine: MPER is a highly conserved region across different strains of HIV and it contains epitopes that are recognized by three broadly neutralizing antibodies (4E10, 2F5 and Z13).[54] By using the short MPER segment peptide instead of a larger envelope fragments, the goal is to avoid the issue of immunodominance and help the immune system recognize the antigen and produce antibodies against it for vaccination.[55]

To elicit broadly neutralizing antibodies such as 4E10, the MPER segment needs to be in a well-defined conformation consisting of two discrete helical segments with a central hinge.[56] The lipid enveloped nanoparticles are used to deliver MPER in a conformation suitable for recognition by broadly neutralizing antibodies. The binding of 2F5 and 4E10 could be enhanced almost two orders of magnitude by the presence of lipid membrane.[57]

5.3 Adjuvant: Monophosphoryl Lipid A

In the Irvine vaccine, to increase the potency of the nano-particles and duration of antibody response, lipid-like adjuvants are incorporated into the lipid envelope. Monophosphoryl Lipid A (MPLA), a derivative of the bacterial cell wall lipopolysaccharide (LPS), is the adjuvant used which can get easily incorporated into the lipid bilayer. This adjuvant helps to increase the

antigen uptake by Dendritic Cells and to enhance the cellular and humoral immune response.[58] MPLA has been chosen as an adjuvant due to its ability to bind to Toll-like receptor 4.[59]

Toll like receptors (TLR) are an important class of proteins which are expressed by both innate and adaptive immune cells. The receptors recognize molecules that are shared broadly by pathogens but are distinguishable from host molecules, bind to them and activate immune cell responses. The pathogen-associated molecules recognized by TLR are usually critical to the pathogen's function and are evolutionary conserved. One of these molecules is the bacterial cell-surface lipopolysaccharides (LPS). While LPS is a complex heterogeneous molecule, its lipid A portion is relatively similar across a wide variety of pathogenic strains of bacteria. Also, MPLA is about 1000 times less toxic than LPS, which makes it a safer material to be used in vaccines.[59,60]

In addition to the useful immunostimulatory and low toxicity properties of MLPA, this adjuvant has been recently approved by the FDA. In October 2009, FDA approved a new adjuvant (ASO4) to be used in an HPV vaccine (Cervarix).[61] ASO4 is a combination of aluminum hydroxide (alum) and monophosphoryl lipid A (MPL), and Cervarix is the first vaccine licensed by the FDA that includes MPLA. In the USA, aluminum salt precipitates (alum) has been used as the only FDA approved adjuvant for the past 70 years and is found in 80% of all vaccines.[62]

5.4 Nano-particle Synthesis

An emulsion–solvent evaporation approach is used to fabricate the lipid-enveloped polymer core nanoparticles as previously reported.[49] Poly(D,L-lactide-co-glycolide) (PLGA) with a 50:50 lactide to glycolide ratio, phospholipids (DOPC, DOPG, maleimide-PEG2k-PE) and MPLA are dissolved in dichloromethane (DCM). The organic solution is then emulsified into de-ionized

water using an IKa T25 homogenizing disperser. Lipids included in the PLGA-containing organic phase enrich at the surface of emulsion droplets.

The enveloped is formed by self-assembly of the lipids around the polymer cores, acting as a surfactant to stabilize the oil-water interface of emulsion. The resulting emulsion is magnetically stirred to evaporate the dichloromethane and form solid PLGA particles with self-assembled lipid layers on their surfaces. The polydisperse particles formed are then separated by centrifugation into cell-sized and virus-sized populations.[49] The desirable particle size would be on the order of 100 nm which is similar to HIV virion size (100nm diameter). This size is desirable for efficient transport of the particles to lymph nodes.[63]

5.5 Nano-Particle Storage

After the lipid envelope nano-particles are synthesized, they will degrade by hydrolysis over time. To prevent degradation, the particles can be lyophilized using a freeze-dryer in the presence of trehalose or sucrose which both act as a cryoprotectant. Addition of cryoprotectant is necessary to maintain the integrity of the particles and protect them during the lyophilization process. It has been shown that, in vaccines using protein antigens, sucrose and trehalose help to stabilize proteins during lyophilization. Also the addition of the excipient prevents aggregation of particles during lyophilization and resuspension.[45] Upon reconstitution, these particles retain a tightly apposed lipid bilayer in saline.

6 Intellectual Property

An intellectual property (IP), such as a patent, serves as a limited monopoly given by the government to the inventor to prevent others from selling, making or using the invention. Patent portfolio is increasingly a critical component of companies' assets.[64] Both product and process patents are common in pharmaceutical industry; however, for biologics, such as vaccines, obtaining a product patent has proven to be difficult. Patents are issued for "inventions", but vaccines are composed of naturally occurring substances which are not "a product of human ingenuity". To make vaccine products patentable, the United States has made an exception that natural substances are patentable if they are "isolated and purified". As a result, there have been several patents issued for naturally occurring DNA and protein biomolecules.[65]

To assess the patentability of the Irvine vaccine technology, the next section will provide a summary of existing relevant patents and patent applications. Following each patent, the distinguishing factor between the Irvine technology and the patent will be discussed. Two essential aspects of the technology analyzed are the use of biodegradable polymer core, lipid enveloped nanoparticles as vaccine delivery systems and the method of attachment of the HIV antigen to the surface of these particles to elicit an immune response.

6.1 Overview of Relevant Patents in HIV vaccine Development

There are several patents on the method of producing microparticles with a biodegradable polymer core and their use in delivering drugs or antigens. The patents on use of polymer-core nanoparticles to deliver drugs mostly go back to 20 years ago and are already expired. For instance, U.S. Patent # 4863735: "Biodegradable Polymeric Drug Delivery System With

Adjuvant Activity" was issued in September 5. 1989, and is no longer protected by Intellectual Property Laws.[66]

U.S. patent number 5,603,960 presents a two step method for producing micro-particles, first by dispersing a bioactive substance, such as an antigen, in a media, such as a biodegradable polymer, and then adding a second medium such as methylene chloride.[67] Upon mixing the two media, phase separation would occur, which results in the formation of microparticles. The patent claims the use of these microparticles for encapsulation of agricultural agents, viruses, cosmetic agents, and particularly immunogens and antigens to induce an immunogenic response. It also explains that the choice of the polymer and the solvent media would depend upon the material to be encapsulated, but it refers to *poly (lactide-co-glycolide)* (PLGA) as a preferred polymer to encapsulate bioactive materials. Similarly, there are many other patents issued on the use of polymer core microparticles to encapsulate drugs or antigens, but in Irvine technology the delivery of the antigen is not via encapsulation; hence, the focus will be on patents describing ways of displaying antigens on the surface.

There are several recent patents issued describing different methods of attachment of the antigens onto the surface of the particles. The U.S. patent number 7,501,134 claims a microparticle composition comprising of a polymer core and polypeptide-containing molecules adsorbed to the microparticle's surface in the absence of all surfactants, including anionic, cationic, nonionic and zwitterionic surfactants.[68] Another claim of the invention is directed to the method of producing the microparticles by a double emulsion (water-in-oil-in-water) or a single emulsion (water-in-oil) free of all surfactants. The polypeptide-containing molecules could be antigens selected from a list including HIV antigens such as HIV gp41 antigen, HIV gp120 antigen, HIV gp140 antigen, HIV p24gag antigen and HIV p55gag antigen. The composition

described can further comprise a biologically active molecule which could be selected from a list of adjuvants including monophosphory lipid A adjuvant (MPLA). These microparticles are stated to have applications in diagnosis of diseases, treatment of diseases, inducing immune responses and vaccines.

The patent discussed above describes microparticles which are similar to Irvine's vaccine particles in terms of the biodegradable polymer core, HIV antigen and MPLA adjuvant used; however, the antigens here are adsorbed to the surface of the particles instead of being covalently bound to it. Also, in Irvine technology, lipids such as DOPC and DOPG are used as surfactants; however, in this patent, the microparticles are formed free of any surfactants which differentiates them from Irvine particles.

U.S. patent number 6,884,435 describes the invention of microparticles with adsorbent surfaces, methods for preparing such microparticles and their uses.[68] The microparticles comprise a biodegradable polymer, a detergent selected from a cationic and an anionic detergent, and antigens adsorbed on the surface of the microparticles. Some claims of the patent are noteworthy and seem to be directly relevant to the Irvine's particles for HIV vaccine. It is claimed that the biodegradable polymer can be poly (D,L-lactide-co-glycolide), and the adsorbed polynucleotide can be an antigen encoding an HIV gp160 polypeptide, an HIV p24gag polypeptide or an HIV p55gag polypeptide. The microparticle can also comprise an adjuvant selected from a list which includes Monophosphory lipid A, and would be an injectable composition capable of eliciting an immune response.

The U.S. patent 6,004,763 describes the use of antigen-carrying microparticles in the induction of humoral or cellular response.[69] The patent does not claim any specific process for making the microparticles; however, it illustrates covalently attaching proteins capable of inducing an

immune response to the surface of the synthetic microparticles. The covalent bond can be formed without a bridging agent or with bridging reagents such as glutaraldehyde or carbodiimide. It is claimed that the microparticle can be composed of a biodegradable polymer core and the surface protein can comprise the B epitope of the gp120 protein of the HIV-1 virus.

Unlike the Irvine nano-particles, the particles described in the patent are not only used to elicit a humoral response. A main focus of the patent is on adjusting the molecular weights of the proteins attached as a way to direct the immune response toward the induction of cellular or humoral response. A molecular weight greater than 30 kD (preferably 50 kD) is said to mainly induce a cellular response, whereas, a protein comprising B and T epitopes with a molecular weight lower than 15 kD induces both humoral and cellular immunity response.

There are other patents issued which discuss use of carriers with adsorbed or entrapped antigens to elicit an immune response. The U.S. patent number 6,855,492 B2 describes a method for enhancing the immunogenicity of an antigen using a submicron oil-in-water emulsion n combination with microparticles which have the antigens entrapped or adsorbed to the surface.[70] The antigen claimed is an HIV antigen and the micro-particle is formed from poly(-hydroxy acid) chosen from the group consisting of poly(L-lactide), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide). Also, US patent application 2007/0275071 A1 is specifically focused on microparticles used for vaccine immunization and antigen delivery for treatment of the HIV virus.[71] It claims that antigens may be fixed or adsorbed to the external ionic or ionisable surface of the microparticles. The microparticles have a water insoluble polymer or copolymer core; however, unlike Irvine's particles, instead of a lipid shell, they have a hydrophilic polymer or copolymer shell with functional groups. The patent application specifics that the antigens could be HIV antigens and the microparticles could be administered to individuals for prevention or

treatment of HIV infections or AIDS. Table 3 is a summary of the patents discussed in this section.

Patent Title	U.S. Patent Number	Date of Filling
Preparation of microparticles and methods of immunization	5,603,960	06/02/1995
Microparticles with adsorbed polypeptide-containing molecules	7,501,134	02/20/ 2003
Microparticles with adsorbent surfaces, methods of making same, and uses thereof	6,884,435 B1	07/29/1999
Antigen-carrying microparticles and their use in the induction of Humoral or Cellular Responses Filed	6,004,763	05/12/1998
Use of microparticles combined with submicron oil-in-water emulsions	6,855,492 B2	08/02/2002
Use of Microparticles for Antigen Delivery	App No: 10/577,974	11/03/2004

Table 3. List of relevant patents to the Irvine Technology

6.2 Assessing the Patentability of the Novel Nano-particle Vaccine

From a careful examination of the existing patents, it becomes apparent that the use of microparticles comprised of biodegradable polymer cores to deliver antigens and induce an immune response is a method commonly used. There are several patents involving the display of HIV antigens on the surface of the particles, the use of MPLA as the adjuvants and biodegradable polymer core microparticles as the delivery system.

The Irvine technology has the additional advantage of the antigen being covalently bound to the lipid membrane. As discussed in the previous sections, the association of the HIV antigen with a lipid membrane, having a composition similar to the virus membrane, helps the antigen to have

the configuration needed to get recognized by the immune system. Also, the Irvine technology is composed of nanoparticles which have a higher surface area and would have a higher antigen: polymer ratio. Furthermore the Irvine nanoparticles specifically use a smaller antigenic segment (only the MPER region) to eliminate the issue of immunedominancy of the other regions of the envelope. Despite some similarities with existing patents, the Irvine vaccine still offers several areas of novelty and is likely a candidate for Intellectual Property protection.

7 Food and Drug Administration (FDA) Licensure Process

One of the most significant challenges in commercializing any new vaccine is to obtain a licensure from U.S. Food and Drug administration (FDA). All new vaccines need proof that they are effective, as well as safe, before they can get approved. To ensure drug's safety and efficacy, the FDA conducts a risk-benefit analysis and determines whether the drug's benefits outweigh its known risks.[72] Once an HIV vaccine has been developed in the laboratory, there are several steps involved before the product can enter the market. The vaccine development phases, from preliminary laboratory research to final product licensure are outlined in the following sections.

7.1 Preclinical Phase and Initial Evaluations

Research Phase

The Irvine technology is currently in the first stage of vaccine development, namely the pre-clinical research phase. For the vaccine to enter human clinical trials, a series of tests in animals needs to be performed. The preclinical and animal testing data is carefully examined to assess whether the product is reasonably safe for initial testing in humans. Once the safety of the vaccine is confirmed, the sponsor or manufacturer must submit an Investigation New Drug (IND) application to the FDA, before it can start any human clinical trials.[73]

Investigational New Drug application (IND)

The IND must contain information in three broad areas: Animal Pharmacology and Toxicology Studies, Manufacturing Information, and Clinical Protocols and Investigator Information. To determine the company's ability to adequately produce and supply consistent batches of the vaccine, FDA will review manufacturing information such as information regarding the composition, the manufacturer, the stability and the controls used for manufacturing of the vaccine.[73] Also, FDA asks for detailed clinical protocols in the IND application to ensure that

humans in the clinical trial would not be subjected to any unnecessary risks. After submitting the IND for the Irvine vaccine, the sponsor must wait 30 calendar days before beginning any clinical trials. During this period, the FDA will review all the information included in the IND to assure the safety of the research population.[74]

7.2 Clinical Development

Phase I

Phase I clinical trials are the first stage of testing the vaccine in humans. This phase is designed to study the metabolism, mechanism of action, safety and side effects of the drug. The population selected for the trials are usually a small (20-100) group of healthy volunteers. These volunteers would be closely monitored to ensure that all the side effects of the drugs are documented. Once the initial safety of the vaccine is confirmed in phase I trails, the phase II trials are performed.[75]

Phase II

Phase II trials are conducted on a larger scale (several hundred people) with the objective of assessing the optimum dose and schedule required to maximize the immune response. These trials are designed to provide data regarding the immunogenicity and efficacy of the vaccine, and may also help to identify the short-term side effects and risks. After phase II trials, vaccines that have been shown to be effective and safe would go through the Phase III clinical trials. Prior to initiation of phase III trials, the FDA recommends that the data from phase II are discussed with the agency to ensure that the phase III trials are designed to provide sufficient data and information needed for obtaining product licensure.[75]

Phase III

Phase III clinical trials enroll a large number of individuals. The population in the trials can range from hundreds to several thousand and depends on factors, such as the incidence of the

infectious disease the vaccine is designed to prevent, and the number of people required for obtaining a valid estimate of the vaccine efficacy. The objective of the Phase III trials is expanded safety and efficacy study of the vaccine. These trials are often randomized meaning that patients are often chosen at random to get either the new vaccine or a placebo. If possible, these studies are also double-blinded, so that neither the doctor nor the patient knows which treatment is being given. Similar to Phase I and II trials, volunteers are monitored closely in this phase and the trial is halted if adverse effects are observed. [75]

7.3 Product Licensing Requirements

Biologics License Application (BLA)

Once the efficacy and safety of the vaccine for human use is established through the clinical trials, a biologics license application (BLA) is submitted to the Food and Drug Administration for approval. The BLA must include all the efficacy and safety data from studies of the vaccine in animals and humans as well as product, manufacturing and control information. The company should ensure that it has the required manufacturing facility before filling for a BLA.[76] For instance, VaxGen, a biopharmaceutical company and vaccine manufacturer, started the second phase *III* trials of its candidate HIV vaccine (AIDSVAX B/B and AIDSVAX B/E) in 2002, but decided to delay its biologics license application for AIDSVAX until 2004 to ensure that the company had all the pre-approval manufacturing requirements.[37]

FDA Advisory Committee Review

The FDA reviews the results from the clinical trials and other relevant information included in the BLA to make a risk/benefit assessment and to either recommend or reject the licensure of the vaccine.[75] Vaccine approval also requires the provision of adequate product labeling. The

vaccine label should include information regarding its proper use and its associated potential benefits and risks. The Vaccines and Related Biological Products Advisory Committee (VRBPAC), a non-FDA expert committee, would review both the findings of FDA and manufacturer regarding the safety and efficacy of the new vaccine and provides advice to the Agency prior to licensure.[74]

Postlicensure Activities

After a vaccine receives permission to enter the market, there will be postmarketing studies (Phase IV trials) to ensure the continuous safety and quality of the product. Phase IV studies also look at the effectiveness of the vaccine and the duration of protection it provides. Information on any rare, serious adverse reactions or side effects that had not been detected in previous trials would be reported to the FDA or the manufacturer. The Vaccine Adverse Event Reporting System (VAERS), a national vaccine safety surveillance program, collects information about adverse side effects. VAERS identifies new safety concerns related to the vaccine and helps to ensure that the benefits of the vaccine continue to be greater than the associated risk. Harmful effects observed in postlicensure phase can stop the vaccine production or restrict the use of the vaccine to certain people.[75] For instance, Vioxx, a nonsteroidal anti-inflammatory drug marketed by Merck & Co, was withdrawn from the market five years after getting licensure due to concerns about the use of drug being associated with increased risk of heart attack and stroke.[77]

8 The Novel Nano-particle HIV Vaccine: FDA Approval Process

The Irvine vaccine is currently in the research pre-clinical stage, and no data from any human clinical trials are available. At this early stage, the assessment of the vaccine overcoming the FDA hurdles can be made only based on the raw materials that the particles are composed of as well as the production method used. At later stages, when sufficient data from animal testing is acquired, an IND application could be submitted and, upon its approval, the testing of the vaccine in human trials can begin. In the section to follow, the composition of the nano-particles and the processing methods are examined from the FDA standpoint.

8.1 Material Composition

Polymer core

The core of the nanoparticles is composed of Poly (lactic-co-glycolic acid) (PLGA), which is a biocompatible and FDA approved polymer. It is commonly used clinically for applications such as resorbable sutures and tissue engineering.[78] It is a biodegradable polymer which degrades into relatively harmless products in the body and has been commonly used in nanoparticles to encapsulate a variety of therapeutic compounds and drugs.[79]

Lipid Envelope

The lipid envelop of the nanoparticles has a composition similar to the lipid shell of the HIV-1 virus as previously reported.[21] It is composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-[phospho-rac(1-glycerol)] (DOPG), and maleimide-PEG2k-PE. There are Good Manufacturing Practice (GMP) versions of all these products currently in the market.[80] GMP refers to the regulations enforced in United States by FDA to ensure that the manufacturing, processing and packaging of the vaccines are monitored and controlled. The objective of these regulations is to minimize instances of error and contamination and to ensure

that the vaccine products are safe, pure, and effective.[81] The existence of several GMP commercial suppliers for all the basic ingredients of the vaccine (the PLGA polymer core and the lipid shell) increases the chances of the technology making it to clinical trials.

Adjuvant

The Monophosphoryl Lipid A (MPLA), an adjuvant used in the vaccine production, is widely being studied and has been used in vaccines in Europe for a long time.[82] Recently, the FDA approved the use of MPLA in United States as well. In October 2009, FDA approved a new vaccine for prevention of cervical cancer (Cervarix) which contains MPLA.[83] The adjuvant in this vaccine is ASO4 which is a combination of aluminum hydroxide and monophosphoryl lipid A. Cervarix is the first vaccine licensed by the FDA that contains MPLA.[83] Before the approval of ASO4, for the past 70 years aluminum salt precipitates (alum) had been used as the only FDA approved adjuvant and is found in 80% of all vaccines.[84] Table 4 lists the adjuvants used in licensed vaccines which were approved in different regions by or before April 2009.[82] Note that many of the livestock adjuvant-vaccine formulations are proprietary and their compositions have not been disclosed.

Humans, US	Humans, United Kingdom, and European Union	Livestock, Worldwide (General Categories)[a]
Aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (alum)	Aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (alum)	Aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (alum)
	Calcium phosphate	Saponin (QS-21)
	MF-59 (Squalene, in Fluad)	Oil emulsions paraffin, mineral oil, lanolin, squalene, ISA-70, Montanide (IMS)
	AS04 (liposome formulation containing MPLA and QS-21) (FENDrix, Cervaix)	Glycerin

Table 4. Vaccine adjuvants used in licensed vaccines (by April 2009). [82] Note that ASO4 is now approved by FDA.

8.2 Processing of the Nano-particles

Apart from the raw ingredients, the FDA might be concerned about the safety and reproducibility of the processing method used to make the nano-particles. Currently the particles are being synthesized in small, "mouse-sized" batches in lab. To scale up this process for human clinical trials, there has to be major revamping of the process as well as quality assurance. Also, the emulsion process of particle synthesis is a kinetic-based self-assembly process rather than an equilibrium process which can result in variations in yield and particle size. Another concern from an FDA standpoint is the use of Dichloromethane (DCM) as the solvent for the organic phase of the synthesis. According to the materials safety data sheet, DCM is toxic and carcinogenic.[85] This highly volatile industrial solvent can be metabolized by the body to carbon monoxide which could lead to carbon monoxide poisoning. [86]

Dichloromethane is currently used as a processing solvent in the extraction of several pharmaceuticals and in the manufacturing of steroids, antibiotics and vitamins.[87] It is also used in processing of tablet coatings; however, there is no dichloromethane left in the coating of the tablet. For the use of DCM in pharmaceuticals manufacturing, the Food and Drug Administration has established residue tolerances.[88] Although Dichloromethane can be used in manufacturing of some pharmaceuticals, the FDA has prohibited its use in some other products such as cosmetics due to DCM's animal carcinogenicity and its likely hazards to human health.[89]

In the current nano-particle synthesis process, the particles are left stirring in an open vessel overnight to drive off the Dichloromethane, but no further measurements are made to ensure that all the DCM has been evaporated. For the vaccine to get approval for being tested in human trials, a rigorous test needs to be developed to ensure that all the Dichloromethane is gone after the particles are fully synthesized.

8.3 Preclinical and Animal Testing

The results of the animal testing of Irvine's particle have shown that the vaccine causes mice to produce very high level of circulating Immunoglobulin G (IgG) antibodies, which are known to be an important mechanism of protection in many types of infections. In terms of efficacy of the vaccine, no challenge studies on the animals have been done yet. The vaccinated animals have not been given a fatal disease to see if the vaccine could save them. For submitting an Investigational New Drug (IND) application, more data needs to be obtained about the efficacy, toxicity, and pharmacokinetics of the vaccine. These data would be presented in the IND to get approval to test the vaccine in human clinical trials.

9 Cost Analysis for the Novel Vaccine

The costs associated with the development and production of a potential HIV vaccine are not easily predictable. A better understanding of the potential costs involved can be obtained by looking at the two major parallel paths involved which include product development and process development. Product development includes going from research to clinical evaluation of the antigen, and process development is the manufacturing and activities required to turn the antigen into a vaccine. Here the cost associated with product development is discussed followed by a discussion of the manufacturing needs in each stage of the development.

9.1 Product Development

The research and development phase of a vaccine production can be very costly and timely. As reported in 2000, the vaccine manufacturers spent about 16% of their proceeds from sales on research and development.[90] Determining the efficacy of the vaccines is usually more difficult and takes a longer time compared to other biologics and pharmaceuticals. Assessing the clinical efficacy of a pharmaceutical is done by examining the drug's effect on eliminating the symptoms in the patient and improving the health conditions. However, since vaccines are used as a preventative measure, their efficacy endpoint is more difficult to determine.[90]

With the new vaccines it is not easy to assess the efficacy at an early stage and usually the efficacy or lack of efficacy of the vaccine candidate is determined at later stages of the clinical trials which results in large expenditures in research.[91] For instance, the HIV vaccine developed by Merck was proven ineffective after going through all stages from research to the phase II clinical trials.[92] Similarly the VaxGen's HIV vaccine trial, a randomized, double-blind, placebo-

controlled, was halted after the phase III of clinical trial.[93] Vaccine efficacy is determined by measuring antibody levels in the serum of vaccinated individuals, or by comparing the disease rates between individuals receiving the vaccine or the placebo after passage of time. This means that there are additional costs associated with monitoring of the patients even years after the vaccine has been administered.[90]

Since vaccines are given to healthy individuals, there are strict safety assessment and requirements for all phases of the trials. Due to FDA's strict safety regulations, the number of people required in the study to ensure the safety of the new vaccines has increased from hundreds to thousands in the past decade to enable detection of the rare side effects and to ensure the safety of the vaccine.[91] For instance, Merck and GlaxoSmith Kline, two competing manufacturers of different rotavirus vaccine have had more than 140,000 participants combined in their preapproval trials.[90] The cost associated with the phase II of the RV144 clinical trial was about 105 million dollars.[94] Conducting any similar HIV vaccine trial would have comparable costs and would be only possible by some support from public institutions.

9.2 Manufacturing

In progression from laboratory discovery to each phase of clinical trials and final licensure, there will be constant changes in processing to scale up and satisfy the greater need for the vaccine production. Lab scale production might be able to satisfy the vaccine needs for pre-clinical studies and maybe phase I trial depending on the processing techniques and equipments needed for manufacturing. Due to strict FDA regulations, in this stage of processing, Good Laboratory Practice (GLP) regulations need to be followed to ensure consistency and safety of the vaccine products. To move on to phase II trials, small scale clinical production is required to enable the

production of a larger amount of vaccines needed for the larger population involved in this trials. In the clinical production, current Good Manufacturing Practice (cGMP) needs to be strictly followed to ensure the mass produced vaccine is bioequivalent.[95]

In phase III trials, pilot scale clinical production is needed which requires development of large cGMP facilities and equipments. Manufacturing development and scaling up can be very costly and in some cases the manufacturing of a product might be abandoned due to manufacturing difficulties and high costs associated with scaling up. Figure 8 shows an illustration of the parallel product and process development.[95]

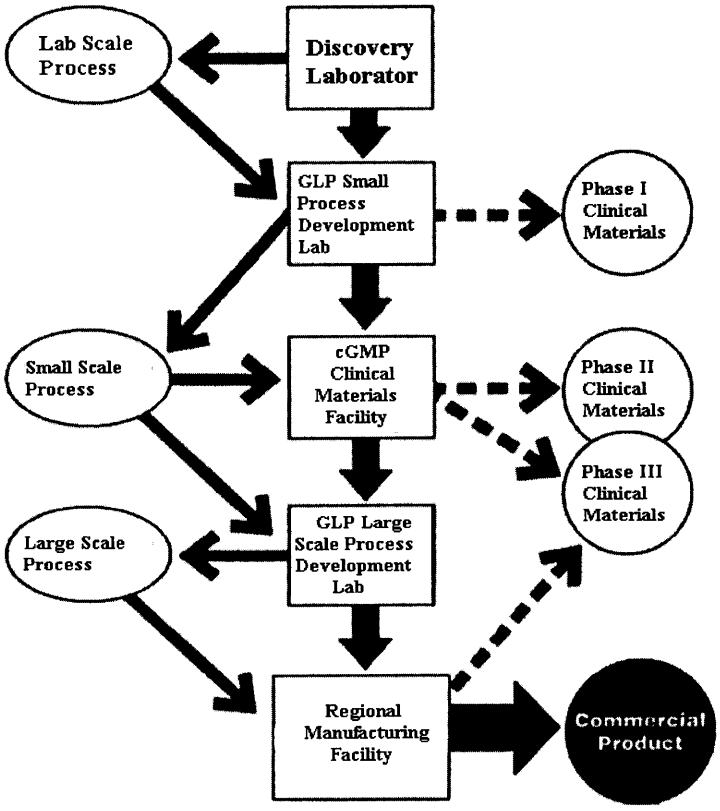

Figure 8: The progression of product and process development from laboratory method and phase I trails, to large-scale manufacturing process and phase III trials, leading to licensed product launch.[95]

As it has been shown, there are several steps involved in going from pre-clinical research to large-scale manufacturing of the vaccine. The exact costs of a vaccine project would highly depend on materials used, processing equipments required and complexity of the manufacturing. Figure 9 displays the cost associated with going from research phase of a vaccine to registration phase.[91] Note that the cost shown here is calculated for an average sized project and relatively straightforward processing, and the cost of developing the Irvine HIV vaccine could be significantly higher than this estimate.

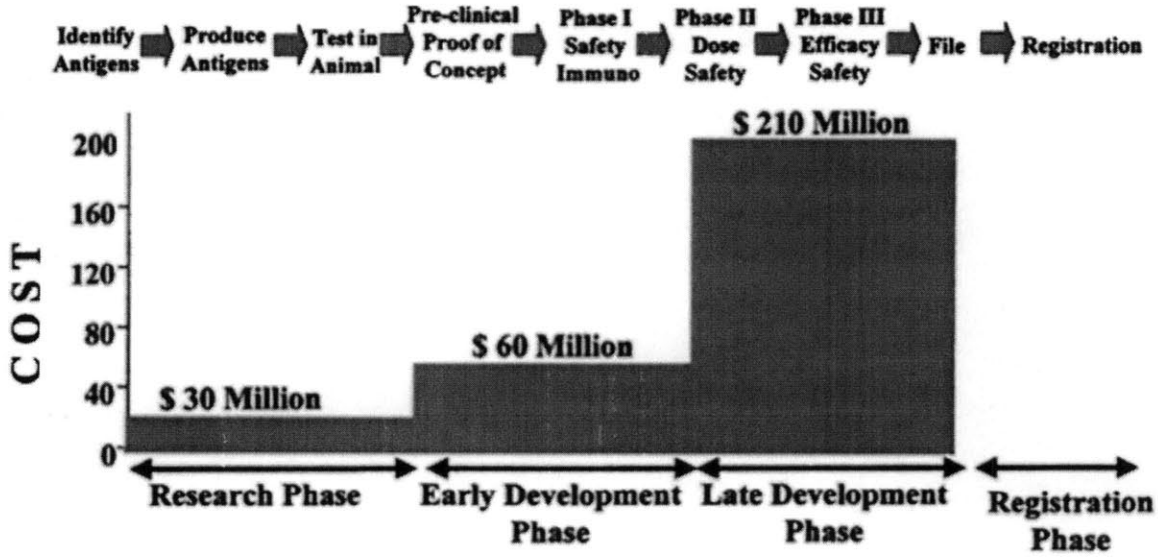

Figure 9 : Development time and cost of a vaccine.[95]

10 Vaccine Supply Chain

10.1 The Cold Chain

Apart from the development and manufacturing of a vaccine, the supply chain plays an equally important role in the vaccine industry. No one would benefit from a vaccine if it is not delivered when it is needed, in good conditions, to the end customers. From manufacturing of a vaccine in a biotechnology company in the United States to administering it in a developing country like Ghana, the vaccine has a long road to travel. The Cold Chain refers to the system that is used to store and transfer the vaccine at the required temperature and in the appropriate conditions from the manufacturer to the recipient.[96] The potency of the vaccine and its immunization effects can be hampered if the vaccine is exposed to excess heat or cold. As shown in Figure 10, the Cold Chain ensures the proper storage and transportation of a vaccine in going from any stage to the next.[96]

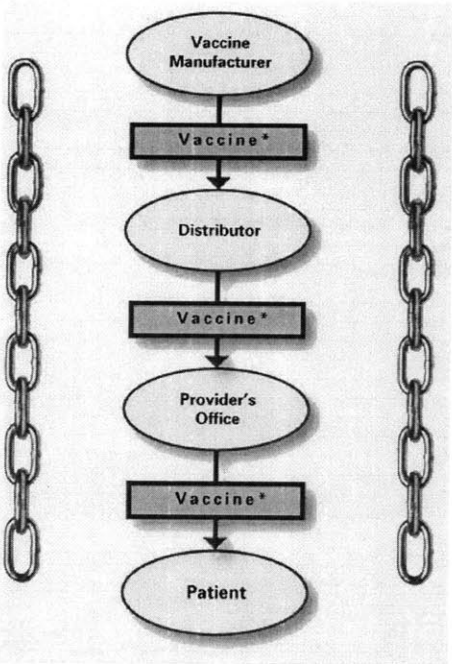

Figure 10: Vaccine Cold Chain. (Vaccine is transported in a refrigerated or frozen state using insulated container or a refrigerated truck)

The cold chain system has been employed for many years despite its high maintenance and waste cost (almost 50% vaccine wasted). [97] It is a method currently used for vaccines that can be cheaply made in large quantities. However, new vaccines, such as a potential Irvine HIV vaccine, can cost 100 times more than original vaccines and any vaccine wasted would result in huge economical costs. In Figure 11 it is shown that the price of the new vaccines is significantly higher per dose compared to the traditional ones and they also have a higher volume per dose. [98]

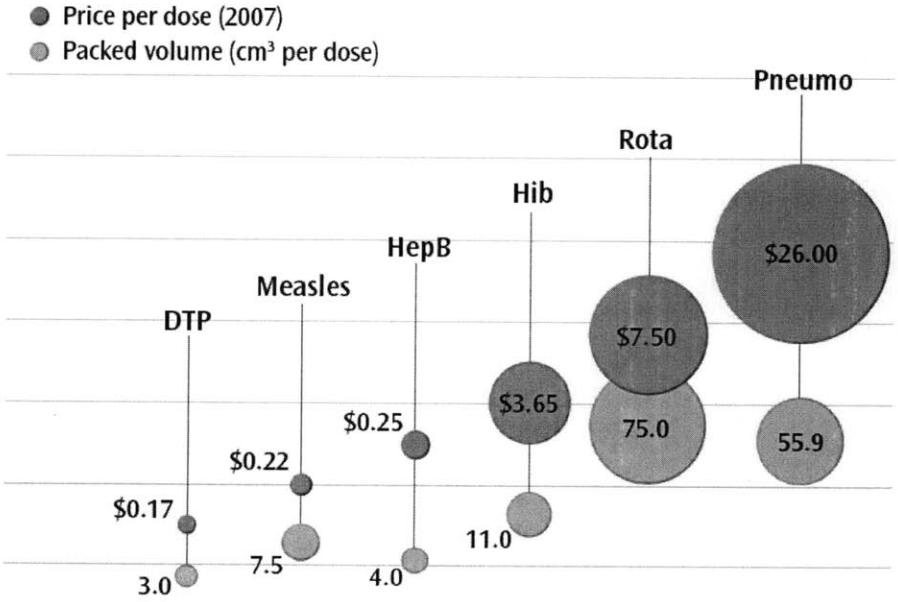

Figure 11: The price per dose and packed volume per dose of new vaccines compared to traditional ones. It can be seen that a dose of a new vaccine such as the one protecting against pneumococcus is more than 100 times that of a vaccine against measles.[98]

To make the supply of the new, costly vaccines feasible, improvements need to be made in the cold chain to reduce the economical costs of vaccine wastage and ensure the safe and proper storage and delivery of vaccines.

10.2 Improving the Vaccine Cold Chain

In recent years, along with the introduction of new vaccines into the market, there have been attempts to use new technologies and innovative ideas to improve the cold chain and reduce vaccine wastage. Organizations such as PATH (Program for Appropriate Technology in Health) have made significant contributions to the improvement of the Cold Chain. Also, there have been on-going projects, such as project Optimize (collaboration between PATH and World Health Organization), with the objective of developing a future supply chain for the efficient and safe supply of the new vaccines.[99] Some of the major improvements which have been made or could be made are discussed in the following section.

Vaccine Vial Monitors

The introduction of the vaccine vial monitors by PATH, in 1996, has been one of the main progresses made in the Cold Chain.[100] The vaccine vial monitor is simply a small sticker (no bigger than a dime) that adheres to the vaccine vial, and changes color upon exposure to heat. Based on the color of the vial, the health workers can determine whether the vaccine is bad or can be safely used for immunization. By eliminating uncertainty about the vaccine conditions, the vaccine vial monitor technology has prevented the disposal of vaccines when there has been uncertainty about the conditions. For instance, in May 2006, due to the earthquake in Yogyakarta, Indonesia, electricity went out at health facilities for several days. The vaccine vial monitors showed that most of the vaccines were usable and undamaged. Hence, a huge economical loss was prevented and 50,000 doses of vaccine, that otherwise would have been disposed, were saved. [100]

In addition to reducing costs due to vaccine wastage, the use of vaccine vials made it possible for the World Health Organization to allow the use of open vials of liquid vaccine for more than a single day which has saved immunization programs millions of dollars worldwide.[100]

Packaging

Improvements to filling and packaging of the vaccines can reduce the chances of contamination and wastage that can happen during transportation. The packaging and filling need to be done in clean room space which is costly to maintain and operate. To reduce the waste of the new expensive vaccines, single-dose presentation or pre-filled disposable syringe devices are recently being used.[101] Although these methods of packaging can cost more and may require more storage space, they reduce the likelihood of contamination. For instance, single dose pre-filled disposable syringes devices such as the BD Uniject are ideal since the syringes are designed such that they can't be re-used or recycled.[102] Although a multi-dose vial would cost less per dose for manufacturing, shipping and storage but the chances of vaccine contamination is more. The reuse of needles or syringes to access the multi-dose vaccine can result in contamination of the whole vaccine and reduce the safety of the procedure.[101]

Storage

Improving the storage and delivery of the vaccines in the supply chain increases the efficiency of the chain and reduces the associated costs. There has been several suggestion made by Project Optimize to improve vaccine storage and delivery. For instance, to reduce cost of vaccine storage in cold conditions, passively cooled carts used to deliver fruits can be used for moving vaccines from one place to the other instead of the traditional vaccine cold boxes.[97] Also, developing battery-free solar refrigerators could minimize vaccine wastage in developing

countries with unreliable power supply. A refrigerator breaking down or its temperature falling below freezing temperature for even a short period of time, can result in thousands of dollars wasted for new vaccines compared to only hundreds of dollars wasted for traditional vaccines.[97] Figure 12 shows that the economical loss as a result of a refrigerator breaking down is about $635.50 for a traditional vaccine such as polio and measles compared to $4, 687.50 cost associated with a new vaccine such as rotavirus vaccine.[98]

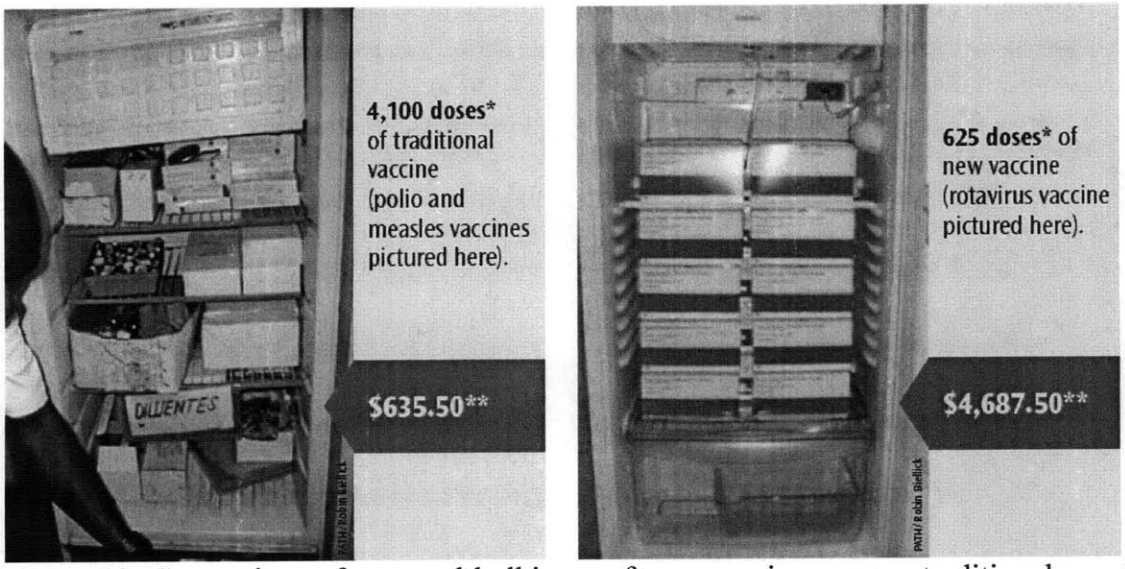

Figure 12: Comparison of cost and bulkiness of new vaccines versus traditional vaccines. New vaccines are bulkier and more costly compared to the traditional ones. A refrigerator full of 4,100 doses of traditional vaccine valued at US$635.50 compared to a refrigerator full of 625 doses of a new vaccine valued at US$4,687.50. [98]

10.3 Novel Nano-particle Vaccine in the Supply Chain

The Irvine vaccine technology would enter the supply chain at the manufacturing segment. The raw materials for the Irvine vaccine particles (lipids, antigens and adjuvants) can be purchased in bulk from companies that use current good manufacturing practice (cGMP). The manufacturing of the particles would take place in Irvine's manufacturing facility (if one is made) or by a

Contract Manufacturing Organization. The manufacturing of the vaccine is only a part of the supply chain and the storing and distribution of the vaccine are both essential parts as well. Since many of the regions in dire need of an HIV vaccine are in Africa (high temperature and humidity), preparing the vaccine in a way that would not require delivery through the cold chain would be extremely advantageous in lowering both the cost and wastage of vaccine.

One novel technology that could be considered to stabilize the Irvine's nanoparticles for storage and transportation is sugar-glass drying which makes the vaccine stable in both tropical temperatures and freezing climates; hence, eliminating the need for refrigerated equipments and the associated costs.[103] The stabilization effects of sugar-glass on Live Poxviral and Adenoviral has been recently investigated. When the viral particles were suspended in solution of disaccharide and were slowly dried at ambient temperature to be coated with an ultrathin film layer of sugar glass, they could be stored at temperatures of up to 113°F for six months without losing their ability to provoke an immune response. However, when they were maintained in liquid storage at 113°F for just one week, one of the two viruses tested was essentially destroyed.[104]

The Irvine vaccine particles made in the laboratory are currently used within 1 or 2 days of production; hence, there is no need to use sugar-glass drying technique to preserve them. However, if the vaccine obtains licensure and needs to be supplied globally, the manufacturer can make the vaccine in the sugar-glass dried form.

Also, as discussed previously, the ability to lyophilize the particles may help avoid the limitations of the Cold Chain encountered in delivery of liquid vaccine formulations requiring refrigeration to developing countries. The lyophilized particles can be stored at room temperature

in a moisture-free container for potentially many months; hence, their delivery and storage would be less costly. The freeze-drying process greatly reduces their water content and prevents the action of microorganism or enzyme which could potentially spoil the vaccine. However, before this vaccine can be administered, the powder must be mixed with the diluents. Upon reconstitution, the vaccine needs to be protected from the sun and heat and should be administered within half an hour.[105]

Lyophilization is frequently used to stabilize labile pharmaceutical agents such as protein and drug-loaded liposomes. For instance, lyophilized cationic lipid-protamine-DNA (LPD) complexes can be stored at room temperature with no significant change in their particle size or loss of transfection efficiency.[106] In another study, it was shown that liposomes containing the adjuvant monophosphoryl lipid A, and bearing maleimide groups at their surface, could be lyophilized and stored without loss of functionality or biological activity.[107] Using lyophilization to make Irvine particles heat-stable would be of paramount importance in making the supply of the vaccine efficient, safe and less costly. In the end, the main goal is to get the vaccine in good conditions to the end customer through an effective supply chain.

11 Market Analysis

11.1 Global Vaccine Market Overview and Growth

The global vaccine market can be divided into three sections: North America (comprising United States and Canada), Europe and The Rest-of-World (ROW). In the global vaccine market, the United States and Europe are thought to be developed markets, regions such as India, China and Brazil are considered to be the emerging markets and Africa is referred to as an under-developed market.[108] In 2008, the North American vaccine market had the largest share of revenue (46%), followed by European market (28.1%), and the Rest-of-World market (25.9%).[109]

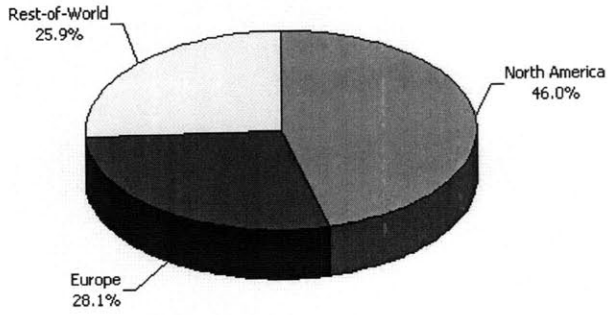

Figure 13 : Vaccine Market Revenue Share by Geographic Region (2008)[109]

The global vaccine market has been expanding; growing from $3 billion in 1992 to $6 billion in 2000 with an annual rate of approximately 9%.[110] In 2008, global vaccine revenues were US $21,280 million, and the compound annual growth rate is expected to be 12.1% from 2008 to 2015.[111] The pharmaceutical and biotechnology industries have become more interested in developing vaccines both as a source of revenue and as a way to beat their competitors. Table 5 shows the percentage of total pharmaceutical revenues created by vaccines. It is clear that there

has been a continuous increase in pharmaceutical industry revenues created by vaccines from 2005 to 2008.[112]

Year	Pharmaceutical Revenues ($ Million)	Vaccine Revenues ($ Million)	Vaccine Revenues (%)
2005	605.0	9.9	1.6
2006	648.0	12.4	1.9
2007	715.0	17.5	2.4
2008	773.1	21.3	2.8

Table 5 : Vaccine Revenues as Percent of Total Pharmaceutical Revenues in 2008 (World)[112]

Market Key Players

The global vaccine market is dominated by the following five companies: Sanofi Pasteur, GlaxoSmithKline (GSK), Merck & Co. Inc., Wyeth, and Novartis. In 2008, 89.8 percent of the market revenues were made by these major players.[113] The revenue shared by these market participants is shown in the Figure 14. Note that "Others" in the Figure includes revenues of companies such as Crucell, CSL, Pfizer, Baxter, Medimmune, Emergent Biosolutions, Sinovac, Solvay and other minor market participants. Wyeth was acquired by Pfizer in October 15, 2009; hence, for 2008, their revenues are listed separately.[113]

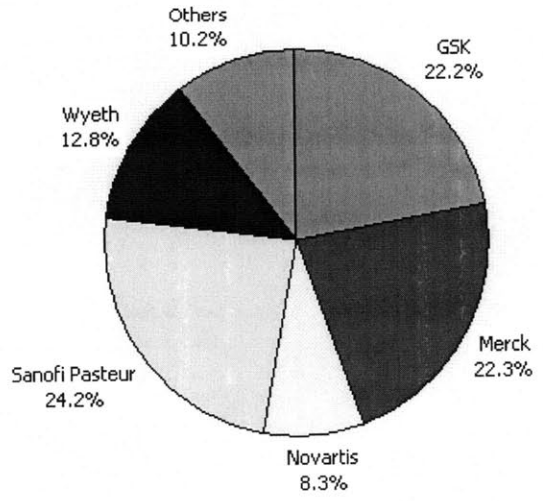

Figure 14: Vaccine Market Revenue Share by Major Market Participant (World), 2008[113]

The five major companies all have a broad portfolio of vaccines, and have been the key players in the market. Since the barriers for entry in the vaccine market are relatively high, the threat of new entrants entering the market is relatively low. Figure 15 shows the historical revenue share trends by the major participants. [113]

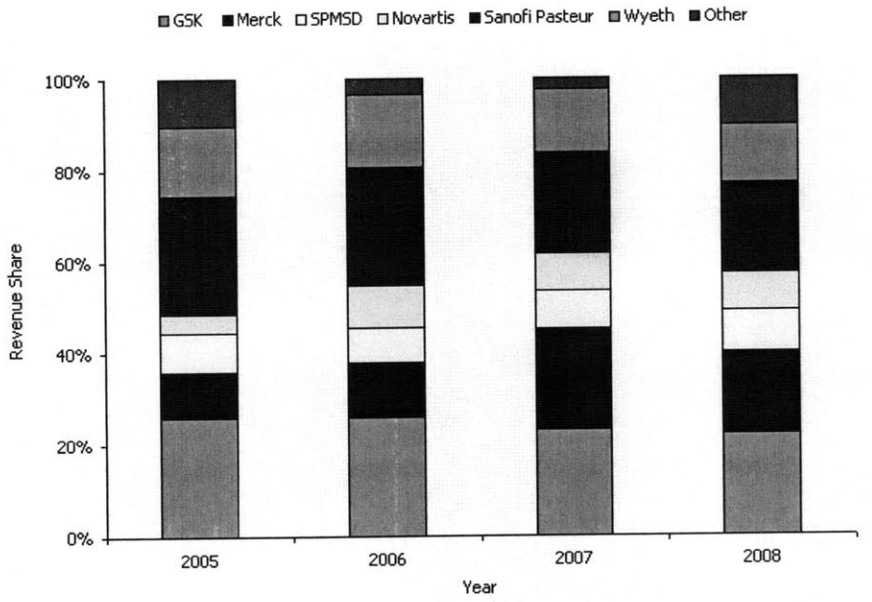

Figure 15: Revenue Share Trends by Different Market Participants (World), 2008 [113]

Other than the major manufacturers, there is another group of companies in the vaccine market, which unlike the first group, do not have a broad portfolio of vaccines, but produce one or two niche products in the market. Many of these tier 2 companies have partnership with tier 1 companies for marketing and manufacturing of their products.[114] The Irvine HIV vaccine would be considered as tier 3 company, since it will be a start-up in its early stages of development.

11.2 HIV Vaccine Market

Currently there is no effective HIV vaccine in the global market and all of the candidate vaccines are in the experimental stage. In 2008, the total global investment in the HIV vaccine research

and development was US $868 million.[115] The public-sector provided 85% (US$731 million) of the funding, while 11% (US$104 million) was financed by the philanthropic sector and 4% (US$33 million) by the commercial sector, namely the pharmaceutical and biotechnology companies.[115] The funding was mainly allocated to five categories including basic research (32%), pre-clinical research (34%), clinical trials (21%), cohort and site development (11%) and advocacy and policy development (<1%). The sources of the global funding and the funding allocation are shown in Figure 16.[115]

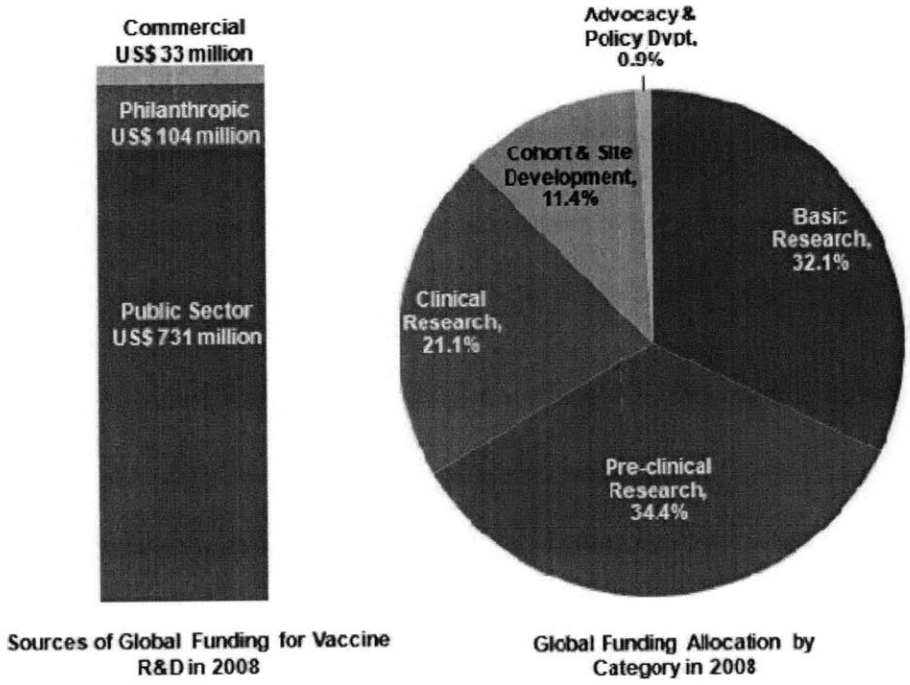

Figure 16: Total HIV vaccine investment US$868 million (2008). The sources and the allocation of the investments are shown.[115]

In the global market, there has been a continuous increase in HIV vaccine expenditure from 2000 to 2007; however, there was a 10% decrease from 2007 to 2008.[115] The trend of HIV vaccine

expenditure in the market, and the allocation of the investments are shown for the years 2000 to 2008 in Figure 17.

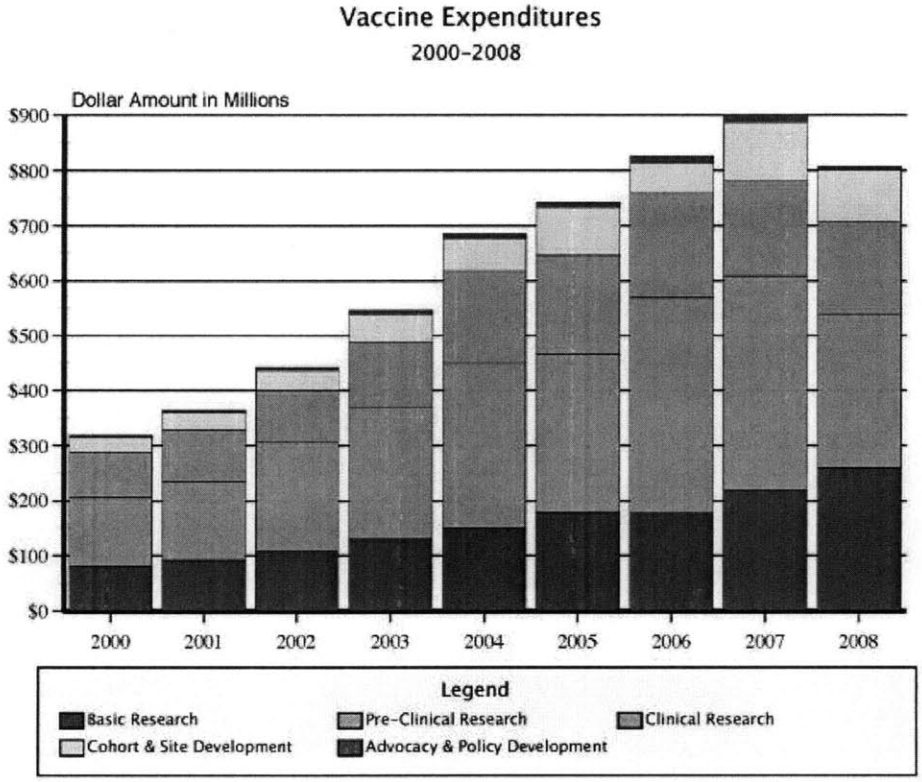

Figure 17: Global Expenditure on HIV Vaccine from 2000-2008[115]

There are several companies developing candidate vaccines against HIV. The preventive vaccine candidates are being tested in HIV-negative people, while the therapeutic vaccines are being tested in HIV-positive people. Some of the vaccines are being studied to see whether they can prevent HIV infection, and some others are examined to see if they lower viral load in people who receive the vaccine before becoming HIV infected. Table 6 includes a list of companies going through different phases of HIV vaccine clinical trials and the technology employed in their vaccine candidate.[116]

Company	Name	Indication (s)	Type
Pfizer	-----	HIV Infections	-----
Novartis	-----	HIV Infections	Recombinant
Sanofi Pasteur	-----	HIV-1 Infections	-----
AlphaVax	-----	HIV-1 Infections	Multigene
Genetic Immunity	DermaVir Patch	HIV-1 Infections	Topical DNA Vaccine
Bavarian Nordic/Pharmexa-Epimmune	-----	HIV-1 Infections	Polytope
Bavarian Nordic	-----	HIV-1 Infections	Multiantigen Vaccine
(pDNA/rRSV)	HIV infections	Prime-Boost Combination Vaccine	-----
GenVec/NIAID	Various Programs	HIV Infections	Adenovactor Serotype Ad35
GSK	HIV	HIV Infections	Recombinant
CytRx	-----	HIV Infections	DNA Vaccine
Inovio	PENNVAX-B	HIV Infections	DNA Vaccine: Treatment/ Prevention
Crucell	-----	HIV Infections	AdVac ® /PER.C6® Technology-Based

Table 6: Selected Product Pipeline for Vaccines in Phase I (World), 2009 [116]

The only vaccine recently tested in Phase III trials was the RV144 which, as discussed in the previous sections, is a prime-boost vaccine combination. As shown in the table, there are several companies and organizations working toward developing an effective HIV vaccine. The search for a vaccine against HIV started in early 1990s and seems to be growing as different types of vaccines are becoming developed and tested.

11.3 Business Model for Novel Nano-Particle Vaccine for Market Entry

To commercialize the Irvine nanoparticles, similar to other vaccines, the discovery needs to be tested in several costly trials, and enough data about the safety and efficacy needs to be gathered before applying for a license. Having financial support and a manufacturing facility are two important requirements for advancing the project from laboratory to the market, and possible paths for fulfilling these requirements are outlined in this section.

Financial Support

The research at the Irvine laboratory is currently funded by the Gates Foundation and the Ragon Institute of MGH, MIT, and Harvard.[117] As discussed in the cost analysis section, to move the project forward strong financial support is required for both conducting the clinical trials and manufacturing of the vaccine. Once laboratory results are sufficient for the vaccine to enter human trials, a small start-up company would be formed. Since the vaccine development is still in an early-stage and has high risks, private investors, including venture capital firms, might be less likely to support the project. Hence, for financial support, an application could be submitted to the U.S. Department of Health and Human Services for the Small Business Innovation Research (SBIR) award.[118]

An SBIR application would be evaluated based on several criteria including the significance of the project and its potential impact, the experience and training of the investigators, the novelty of approaches and methodologies of the project, and the justification of the clinical research.[118] The preclinical development and evaluation of HIV vaccines, adjuvants, and delivery systems are Small Business High-Priority Areas of Interest[119]; hence, once an application is proven to satisfy the criteria above, it is likely that it will receive funding.

In general, funding for HIV vaccine research is mainly provided by the governmental agencies and philanthropic organizations. Table 7 outlines the HIV-1 vaccine efficacy studies done to date and their source of funding. The funding for the recent RV144 HIV vaccine trial was partially (75%) provided by US National Institute of Allergy and Infectious Diseases (NIAID) and (25%)

by the US Army.[94] Similarly for the Irvine vaccine, there would be a need for significant financial support from government and/or philanthropic organizations.

Vaccine Developer	Study	Status	Funding provider
VaxGen	VAX 003, VAX 004 (phase 3)	Completed in 2003 (source is*)	VaxGen[120]
Merck	HVTN 502 STEP, HVTN 503 Phambili (phase 2b)	Stopped in 2007 (source b)	Merck and HIV Vaccine Trials Network (supported by National Institute of Health)[121]
Sanofi, VaxGen (GSID)	RV 144 (phase 3)	Completed in 2009	National Institute of Allergy and Infectious Diseases and US Army[122]
NIH VRC	HVTN 505 (phase 2)	Started in 2009	National Institute of Allergy and Infectious Diseases (NIAID)[123]

Table 7: HIV-1 Vaccine Efficacy Studies

Manufacturing

In the development of the Irvine vaccine, it would be too costly to construct and design manufacturing facilities specifically for the particles synthesis. Companies such as Merck or VaxGen may be at an advantage compared to the Irvine startup since they already have manufacturing facilities which they could use to make their candidate vaccines. For clinical materials manufacturing and large-scale manufacturing of the Irvine vaccine a contract manufacturing organization (CMO) should be considered.[124]

There are several contract manufacturing organizations that the startup could potentially work with. For instance, SynCo Bio Partners B.V., the cGMP manufacturer of clinical and commercial biopharmaceuticals, is a contract manufacturing organization in Amsterdam. This organization won the Vaccine Industry Excellence (ViE) award for Best Contract Manufacturing Organization (CMO) at the Washington World Vaccine Congress in 2009.[125] Diosynth Biotechnology is

another global Contract Manufacturing Organization (CMO) specializing in current Good Manufacturing Practices (cGMP) Biopharmaceutical production.[115] Working with a CMO would accelerate the timeline between the discovery of the vaccine and when it is introduced to the world and would require lower initial capital investment, thus minimizing the project's cost.[124]

11.4 Business Opportunities for Novel Nano-particles other than HIV Vaccine

The lipid enveloped polymer core nano-particle technology developed by the Irvine group can potentially have several applications. So far, the focus has been on the use of these particles for a preventive HIV vaccine; however, these particles can have other application as discussed in the following section.

Vaccine Applications

The nano-particles used in the Irvine HIV vaccine project can potentially be used in development of other types of vaccines. The main structure of the particles could stay the same, but the antigen displayed on the surface of the particles would have to change to be specific to the disease of interest. These nano-particles, by efficiently displaying the antigens on their surfaces, may greatly lower the dose of antigen needed to achieve immunity; and hence could potentially help reduce the cost of the vaccines. Bioactive molecules can be displayed on the surface of these particles either by direct incorporation into the lipid bilayer or by covalent conjugation via a reactive group, such as sulfhydryl group or primary amine group.

Drug Delivery Applications

Other than vaccine applications, the nanoparticles could be used as drug delivery system. Lipids with functionalized headgroups which are incorporated into the lipid bilayer could bind to different types of drugs. Also, nanoparticles or microparticles having a biodegradable polymer core, can be used as controlled drug release systems. As the polymer goes through hydrolysis

and degrades, it can release drugs in the body. It has been shown that the time required for degradation of PLGA is related to the ratio of the monomers (lactic/glycolide) used in production; hence, by controlling this ratio, the desired drug release rate could be obtained.

Using PLGA can be extremely helpful to optimize the therapeutic effects of drugs. For any drug, there is a concentration range over which the drug is considered to have optimal therapeutic effects in the body, referred to as "therapeutic window". Concentrations of the drug below this range have no therapeutic effects and above this range may have undesired toxic effects.[127] The PLGA polymer degradation can be controlled to release drug in the body over time ensuring that the concentration always remains in the therapeutic range. For instance, Lupron Depot used for treatment of prostate cancer contains the anticancer drug leuprorelin acetate within a poly(lactic-co-glycolic acid) (PLGA) matrix. Table 8 lists some examples of products commercially available in the market that use polymer cores.

Drug	Trade name	Company	Application
Leuprorelin acetate	Lupron Depot	Takeda	Prostate cancer
Leuprorelin acetate	Trenantone	Takeda	Prostate cancer
Recombinant human growth hormone	Nutropin depot	Genentech-Alkermes	Growth hormone deficiency
Goserelin acetate	Zoladex	I.C.I.	Prostate cancer
Octreotide acetate	Sandostatin LAR depot	Novartis	GH suppression anticancer
Triptorelin	Decapeptyl	Debiopharm	Cancer
Recombinant bovine somatropin	Posilac	Monsanto	Milk production in cattle
Risperidone	Risperdal Consta	Janssen	Schizophrenia

Table 8: Examples for pharmaceutical products based on drug-loaded, biodegradable microparticles.[127]

Encapsulating drugs in the polymer core is also advantageous since it enables the injection of the drug directly into the desired tissue.[127] As a result, the concentration of the drug in other parts of the body and the negative side effects associated with it could be minimized. This is especially useful for highly potent drugs such as anti-cancer drugs and neurotrophic factors which can cause serious toxic effects in the body.

The controlled release microparticles can be used for administration of anti-cancer drugs to parts of the brain which are not accessible to the surgeon without causing damage to the organ. They can also be used in cell-therapy as well to optimize the growth and differentiation of cells. Cells would adhere to the microparticles and the drug released from the particles enhances cells' differentiation and growth. The PLGA drug-loaded microparticles have been used to improve differentiation of cells which produce dopamine, which is used in treatment of Parkinson's disease.[127]

The Irvine polymer core nanoparticles could not be used in the development of a preventive HIV-1 vaccine, but could also be used in production of other types of vaccines. Moreover, the biodegradable polymer core nano-particles have applications in drug delivery systems. Overall, there are plenty of potential applications and business opportunities for the Irvine novel technology.

12 Conclusion

There is tremendous amount of research going on to identify a vaccine against Human Immunodeficiency Virus. Several candidate HIV vaccines have been studied in clinical trials; however, there is still no global HIV vaccine to help end the epidemic. In this paper, the technological and economical aspects of lipid-enveloped nano-particles for a potential HIV vaccine are evaluated. The particles, created by the Irvine laboratory, are designed to elicit humoral immunity response and are used both as antigen delivery systems and adjuvants. The technology is currently at pre-clinical research stage, and has not yet been tested in human clinical trials.

The results of an extensive patent search indicates that, despite some similarities between the Irvine technology and existing patents, the nano-particle vaccine offers several areas of novelty and is likely a patentable technology. Since the Irvine vaccine is in the pre-clinical research stage, to evaluate the possibility of the vaccine overcoming the FDA hurdles, the paper has focused on the materials used in the vaccine and the method of production. There is no data available from clinical trails to be used in our assessment, but if the vaccine shows efficacy and safety in humans, it's implementation in the market could be costly.

Going from pre-clinical research to large-scale manufacturing of the Irvine vaccine will involve several steps and the cost would mainly depend on the materials used, processing techniques and the clinical trails required to obtain FDA approval. In the early development of the vaccine, it will be too costly to construct and design manufacturing facilities specifically for the Irvine vaccine; hence, using a contract manufacturing organization has been suggested. To minimize the cost of supply of the vaccine, technologies such as Lyophilization could be used to enable the

vaccine to avoid supply through the cold chain. Improving the storage and delivery of the Irvine vaccine will increase the efficiency of the chain and reduce the associated costs.

The use of these nano-particles is not limited to the development of an HIV vaccine. There are several business opportunities for the Irvine nano-particles. By modifying the antigen displayed on the surface of the particles, the Irvine technology can be used in design of vaccines for other diseases. Furthermore, nano-particles with a biodegradable polymer core can be used as controlled drug release systems in drug delivery applications. Thus, in addition to a potential HIV vaccine, there are several other applications for the Irvine technology.

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